The Impact of the Micro-Lightguide O2C for the Quantification of Tissue Ischemia in Diabetic Foot Ulcers

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OBJECTIVE — Tissue oxygen supply is crucial for wound healing. Especially in diabetic foot lesions, the chances for healing are mainly dependent on the presence or absence of ischemia. This study investigates the impact of the tissue O2 analysis system "O2C" for noninvasive quantification of tissue oxygenation in diabetic foot ulcer patients.

RESEARCH DESIGN AND METHODS — O2C assessed relative blood flow (flow), flow velocity (velo), and hemoglobin concentration (rHb) and hemoglobin oxygenation (SO_2) at 2 and 6 mm depth (means ± SE). *1*) Measurements were performed on intact skin of the forefoot and forearm of 20 healthy volunteers on 2 consecutive days. *2*) Parameters were assessed on intact skin of the forefoot of diabetic foot ulcer patients ($n = 14$). *3*) Measurements were performed directly at the wound site in diabetic patients ($n = 14$).

RESULTS — *1*) Flow, velo, rHb, and SO_2 at 2 and 6 mm depth were not significantly different when measured at 2 consecutive days. *2*) There were no significant differences between diabetic subjects and healthy volunteers. Only flow in 6 mm depth was significantly higher in diabetic subjects (75 ± 13 vs. 51 ± 0.4 arbitrary units [AU], $P < 0.05$). When diabetic foot ulcer patients were split into healers and nonhealers, initial readings of SO_2 at 2 mm (32 ± 6 vs. 44 ± 3%, $P < 0.05$) and flow in 6 mm (28 ± 1 vs. 51 ± 0.6 AU, $P < 0.05$) were significantly reduced in nonhealers compared with control subjects, whereas in healers flow in 6 mm (70 ± 0.6 vs. 51 ± 0.6 AU, $P < 0.05$) was significantly higher than that in control subjects. *3*) Initial SO_2, rHb, flow, and velo were significantly lower in nonhealing compared with healing wounds.

CONCLUSIONS — O2C is a new reliable and valid method for noninvasive measurement of tissue oxygenation and microvascular blood flow in patients with diabetic foot ulcers.

Diabetes Care 27:2863–2867, 2004

Foot ulcers are severe long-term complications in diabetic patients. In addition to local wound therapy, adequate tissue oxygenation is crucial for healing since oxygen is necessary for collagen formation, bactericidal activity of neutrophils, and endothelial cell function. Prognosis of diabetic foot lesions is mainly dependent on the presence or absence of tissue ischemia. However, quantitative assessment of peripheral perfusion and microcirculation is still challenging. Different methods like Doppler ultrasound or angiography are able to evaluate macrocirculation, but a reliable, easy technique to assess microcirculation, i.e., tissue perfusion, is not available. Previous studies have shown that healing might be predictable by measurement of transcutaneous oxygen tension ($TcPO_2$) (1–3). If $TcPO_2$ is <30 mmHg, complete healing cannot be expected (4). However, $TcPO_2$ is only a qualitative, not a quantitative, approach for evaluating peripheral perfusion and has a main limitation due to the need for heating the skin before measurement. This affects the resistance of skin vasculature and attenuates reflex mechanisms.

Lightguide tissue spectrophotometry (O2C), a combination of laser Doppler spectroscopy and tissue spectrometry, is a relatively new approach for evaluating tissue oxygen supply (5). In previous investigations, this system was used to demonstrate the effect of postural changes in patients with chronic venous insufficiency (6) and for the early identification of diabetic foot ulcers that may require intervention (7). In this study, we correlated wound tissue microperfusion of diabetic ulcers measured by O2C with clinical healing.

RESEARCH DESIGN AND METHODS

A total of 34 subjects was studied. The first group consisted of 20 healthy volunteers (mean age 42 years [range 21–52]) from the medical staff. During July through October 2003, the second group ($n = 14$) was randomly selected from patients (mean age 68 years [59–78]) attending for the first time the ambulatory wound clinic at the Department of Surgery of the University Hospital Tübingen.

These patients were classified as ischemic or nonischemic, depending on clinical examination, ankle-brachial pressure index measured by a handheld Doppler (5 MHz), and transcutaneous oxygen tension ($TcPO_2$) on the dorsum of the feet (TCM 4 Series; Radiometer, Copenhagen, Denmark). Patients were included in the study if the following criteria were present: nonpalpable peripheral pulses and a $TcPO_2$ value <30 mmHg. None of these patients suffered from severe neuropathy according to the clinical component of the Michigan Diabetic Neuropathy Score (8). All of these patients had Wagner grade I ulcers (9). Ulcers were located on the plantar surface of the foot and treated with moist wound dressings. Prescription footwear was used for pressure relief. The area of the ulcer was assessed by digital photoplanimetry.

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Received for publication 15 June 2004 and accepted in revised form 26 August 2004.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

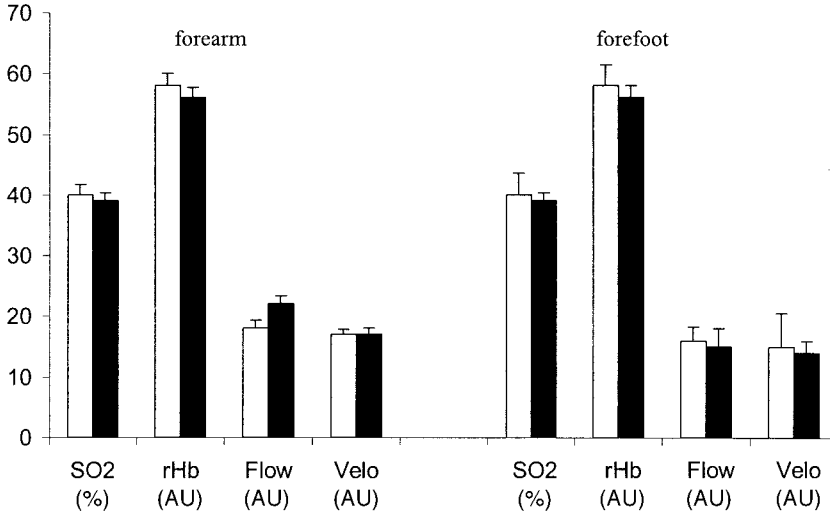

Figure 1—*O2C measurements were performed on the forefoot and forearm of healthy volunteers (n = 20) on 2 consecutive days at 2 mm depth. □, day 1 measurement; ■, day 2 measurement. P < 0.05 was considered significant.*

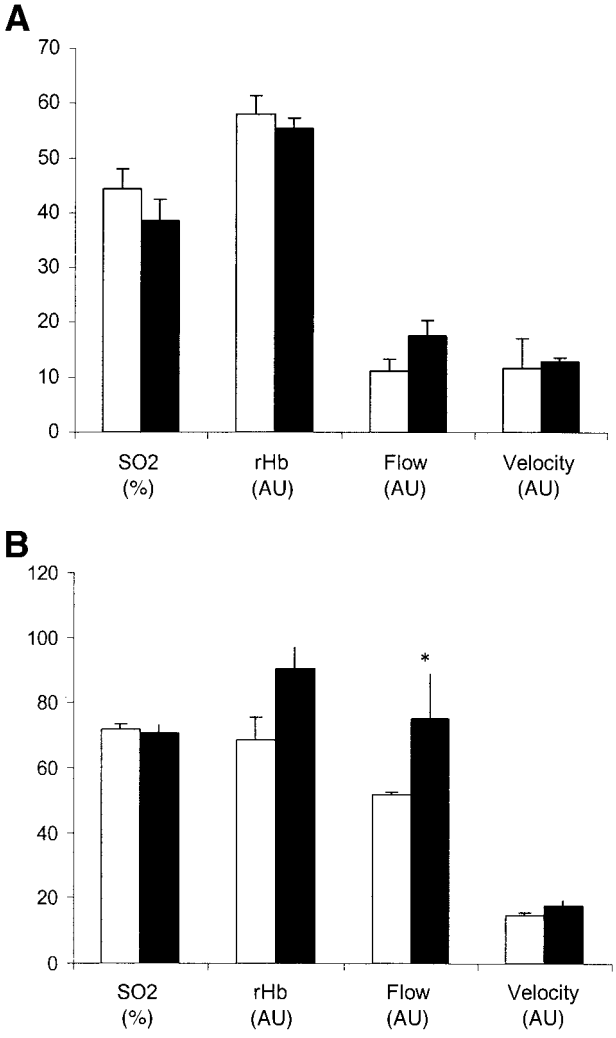

Figure 2—*O2C measurement on healthy skin at the forefoot of healthy volunteers (□; n = 20) and diabetic patients (■; n = 14). A: Measurements were performed at 2 mm depth. B: Measurements were performed at 6 mm depth. P < 0.05 was considered significant.*

Measurements were carried out at the initial visit in an air-conditioned room heated to 22°C after debridement. If bleeding occurred during debridement, measurements were made after hemostasis. No wound demonstrated clinical signs of infection at the beginning of the study or during follow-up. All patients had been acclimated for at least 15 min and brought into the supine position. Legs were covered with a blanket to avoid vasoconstriction through cold. Skin was covered with opsite transparent film to avoid crossinfection. The O2C fiber probe was held in place at the wound site through gentle pressure by a leukosilk tape.

Micro-lightguide spectrophotometer (O2C)

The optical method for measuring both relative blood flow by the laser Doppler technique and hemoglobin oxygenation and hemoglobin amount in tissue by spectrometric techniques was described in detail in previous studies (10). Determination of hemoglobin was first described by Krug (11), while the principle of relative blood flow measurement was detailed by Shepherd and Öberg (12) and Bonner and Nossal (13) and specifically described for the applied device by Ott et al. (14). The micro-lightguide spectrophotometer O2C (Oxygen to See; LEA Medizintechnik, Gießen, Germany) transmits continuous wave laser light (830 nm and 30 mW) and white light (20 W, 500–800 nm, and 1 nm resolution) to tissue where it is scattered and collected on the skin surface at fibers in the probe. The collected light is split into its spectral components by charge-coupled device array and converted into an electrical signal. The digitized signal is recorded on a personal computer. Data are analyzed by comparison with prerecorded desoxygenated and oxygenated hemoglobin spectra.

A Doppler shift effect is caused by movement of erythrocytes. This Doppler shift of the illuminated laser light is detected, analyzed, and displayed as blood flow velocity (velo). The detected laser signal also correlates with the number of moving erythrocytes in tissue. The product of moving erythrocytes times velocity of each erythrocyte is used for the calculation of relative blood flow (flow).

White light is used for the detection of hemoglobin parameter: oxygen saturation (SO_2) and relative amount of hemo-

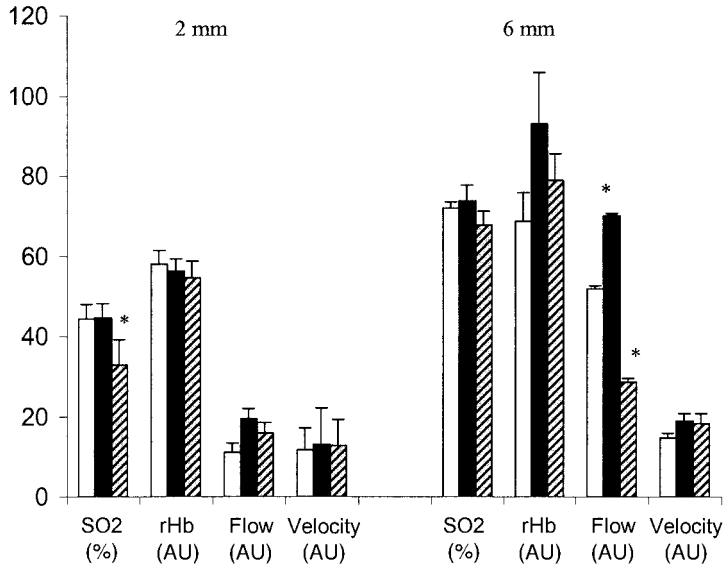

Figure 3—*O2C measurements were performed at the forefoot of diabetic patients at depths of 2 and 6 mm. Diabetic patients were divided into two groups, healers (■; n = 6) and nonhealers (▨; n = 8). □, healthy volunteers* (n = *20). P < 0.05 was considered significant.*

globin (rHb). The oxygen saturation is determined by the color of blood. The tissue hemoglobin value is determined by the amount of light absorbed by tissue. This measurement represents a hemoglobin concentration per tissue volume and is independent from the vessel density (microvessels), vessel lumen (only vessels <100 μm), and hemoglobin quantity in the blood.

Measurement schedule

In the first setting, measurements were performed on intact skin at the forefoot of healthy volunteers ($n = 20$) to detect SO_2, rHb, flow, and velo at 2 and 6 mm depth. On the following day, measurements were repeated at the same location and compared with the results from the day before.

In the second setting, measurements were performed in patients with diabetic foot ulcers ($n = 14$) both on intact skin at the forefoot and at the wound site in the center of the wound. Patients were then prospectively assessed using a standardized wound-documentation system (15). At the start of the study, reduction in wound size ≥50% during 6 weeks was defined as the end point of the investigation, dividing patients into two groups. Group 1 consisted of diabetic patients with ulcers that healed during therapy (healers) and group 2 of diabetic patients with nonhealing ulcers (nonhealers).

Statistical analysis

All data are presented as means ± SE. Differences between each groups were calculated by the Wilcoxon-Mann-Whitney *U* test. A *P* value <0.05 was considered significant. For multiple comparisons, values were adjusted according to Bonferroni.

RESULTS

Healthy volunteers

Figure 1 shows values measured on intact skin at the forefoot and forearm of healthy volunteers at 2 mm depth on 2 consecutive days. SO_2, rHb, flow, and velo were assessed. Values measured on both days were not significantly different.

Diabetic patients

Measurement on intact skin at the forefoot. There were no significant differences between diabetic patients and volunteers when measured at 2 mm depth (Fig. 2*A*). However, at 6 mm depth, diabetic patients had a significantly higher flow than healthy subjects (Fig. 2*B*).

Diabetic patients were divided into two groups. In group 1, ulcers healed during therapy (healers), whereas in group 2, wounds did not heal (nonhealers).

Measured at 2 mm depth, nonhealers showed significantly reduced SO_2 compared with healthy control subjects and healers. However, there was no difference between control subjects and healers. Additionally, there was no difference at 2 mm among control subjects, healers, and nonhealers concerning rHb, flow, and velo.

At a depth of 6 mm, healers had a significantly higher flow compared with control subjects, whereas nonhealers had a significantly lower flow compared with control subjects. However, there were no differences concerning all other parameters, especially SO_2, which was not markedly different among the groups (Fig. 3).

Measurement at the wound site. At 2 and 6 mm depth, healers had significantly higher values in SO_2, rHb, flow, and velo compared with nonhealers (Fig. 4).

Demographic data. There was no significant difference in wound size between healers (3.69 ± 1.42 cm^2) and nonhealers (3.4 ± 1.46) at the initial visit. Additionally, age (68 ± 8 vs. 69 ± 9 years), neuropathy score (16.14 ± 3.8 vs. 14.57 ± 3.6), and $TcPO_2$ values (27 ± 6.0 vs. 19 ± 7.3 mmHg) did not show significant differences between healers and nonhealers.

CONCLUSIONS

— Adequate tissue microperfusion is essential for proper wound healing. Physical examination of peripheral pulses or the ankle-brachial pressure index may be inaccurate, especially in diabetic patients. Evaluation of microcirculation is difficult, and available methods have serious limitations. In diabetic patients, measurement of $TcPO_2$ was used as a helpful tool to assess tissue oxygen supply and predict chances for sufficient tissue repair (16,17). However, measurement of $TcPO_2$ is technically difficult, time consuming (18), and not possible in the most relevant area, the wound itself.

With this study using the microlight-guide-spectrophotometer O2C, we describe for the first time a quantitative method to assess microperfusion directly at the wound site. This makes it possible to predict the severity of ischemia and chances for healing and to select the appropriate treatment.

The first goal was to prove that O2C produces reliable and reproducible data. One major advantage of O2C is frequent measurements. O2C measures every other second. Averaging a lot of single measurements results in much more accurate data. The parameters SO_2, rHb, flow, and velo, measured in 20 healthy

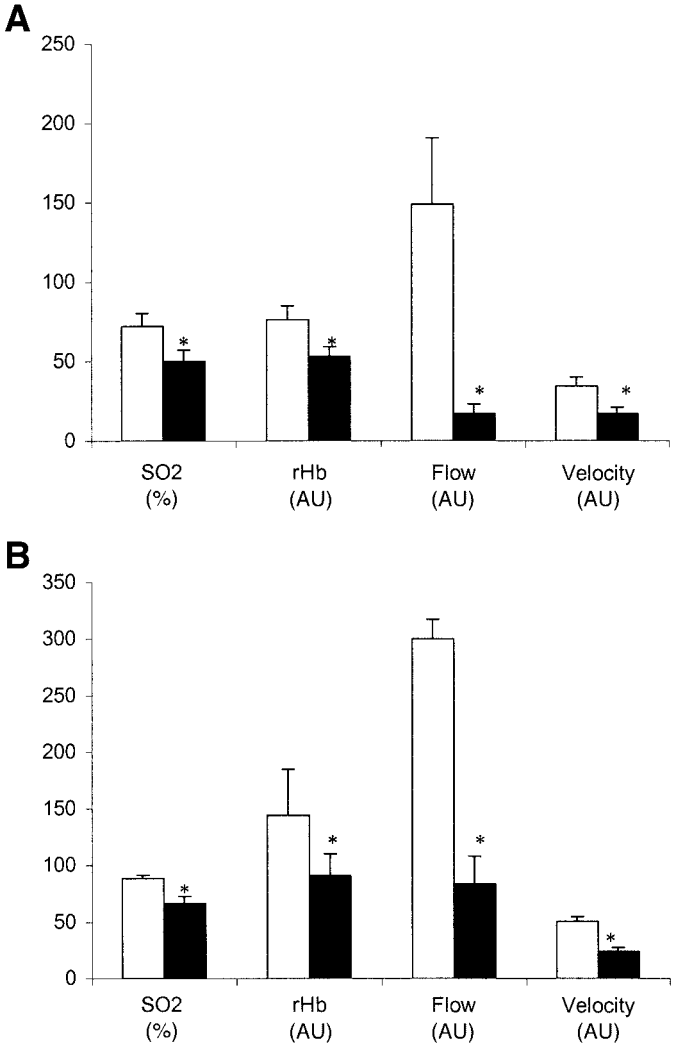

Figure 4—*O2C measurement at the wound site. Wounds were classified as healing (□; n = 6) or nonhealing (■; n = 8).* A*: Measurement was performed at 2 mm depth.* B*: Measurement was performed at 6 mm depth.* $P < 0.05$ *was considered significant.*

volunteers on 2 consecutive days on intact skin at either the forefoot or forearm, were not significantly different.

All diabetic patients included in this investigation did not have palpable peripheral pulses at the initial visit. However, SO_2 measured at the forefoot at 2 and 6 mm depth did not show any difference compared with healthy control subjects, while flow detected at 6 mm was even higher in diabetic patients. At first sight, this seems to be contradictive. But when these patients were divided into two groups, healers and nonhealers, it was apparent that patients with wounds that did not heal during treatment had a significantly reduced SO_2, whereas patients with wounds that did heal showed no difference in SO_2 compared with control subjects. These findings are in concordance with Rajbhandari et al. (7), who could show that SO_2 is reduced in diabetic patients with neuroischemic compared with neuropathic ulcers. Similarily, flow is significantly higher in healers compared with nonhealers, while flow in nonhealers is even lower compared with healthy control subjects. This provides further evidence that SO_2 (2 mm) and flow (6 mm) are predictive parameters to assess chances for complete healing. It seems that 30% is a threshold for SO_2 measured on intact skin at the forefoot at 2 mm depth. Below 30% healing cannot be expected. Similarily, a flow of <30 arbitrary units, measured at 6 mm depth, indicates severe impairment of healing.

Measurements directly at the wound surface show significantly higher values for healers regarding SO_2, rHb, flow, and velo. At 2 mm depth, flow is ~10 times higher and, at 6 mm depth, ~4 times higher in healers than in nonhealers. However, the major problem is a lacking control value, since healthy volunteers do not have wounds. This makes it important to combine measurements at the wound site with measurements on intact skin at the forefoot.

The O2C micro-lightguide spectrophotometer is a new promising tool for the quantitative assessment of tissue microperfusion. Measurements are easy to perform and not time consuming. Results are accurate since values are assessed every other second. It might now be possible to detect clinically relevant ischemia earlier, predict the future healing process, and choose a more appropriate treatment schedule.

Acknowledgments— This work was supported by funds from the University of Tübingen AKF-Grant (95-0-0).

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